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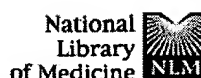
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Molecular and evolutionary relationships among enteric bacteria.

Lawrence JG, Ochman H, Hartl DL.

Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110.

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Classification of bacterial species into genera has traditionally relied upon variation in phenotypic characteristics. However, these phenotypes often have a multifactorial genetic basis, making unambiguous taxonomic placement of new species difficult. By designing evolutionarily conserved oligonucleotide primers, it is possible to amplify homologous regions of genes in diverse taxa using the polymerase chain reaction and determine their nucleotide sequences. We have constructed a phylogeny of some enteric bacteria, including five species classified as members of the genus *Escherichia*, based on nucleotide sequence variation at the loci encoding glyceraldehyde-3-phosphate dehydrogenase and outer membrane protein 3A, and compared this genealogy with the relationships inferred by biotyping. The DNA sequences of these genes defined congruent and robust phylogenetic trees indicating that they are an accurate reflection of the evolutionary history of the bacterial species. The five species of *Escherichia* were found to be distantly related and, contrary to their placement in the same genus, do not form a monophyletic group. These data provide a framework which allows the relationships of additional species of enteric bacteria to be inferred. These procedures have general applicability for analysis of the classification, evolution, and epidemiology of bacterial taxa.

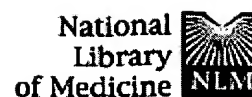
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Biological activities of native and recombinant *Borrelia burgdorferi* outer surface protein A: dependence on lipid modification.

Weis JJ, Ma Y, Erdile LF.

Department of Pathology, University of Utah School of Medicine, Salt Lake City 84132.

Borrelia burgdorferi lipoproteins are 50- to 500-fold more active as cytokine inducers and B-cell mitogens than *Escherichia coli* lipoproteins and synthetic peptides containing the tripalmitoyl-S-glycerol-cysteine moiety. To investigate the source of this unique potency, we compared native OspA from *B. burgdorferi* with recombinant lipidated OspA produced in *E. coli*. As little as 10 ng of either protein per ml stimulated B-cell proliferation and production of cytokines and nitric oxide by macrophages. The two proteins induced comparable antibody responses in mice. Nonlipidated OspA made in *E. coli* had no stimulatory activity. Thus, lipid modification is essential both in vivo and in vitro for the immunological properties of OspA. The lipid moiety appears equally active whether produced in *B. burgdorferi* or in *E. coli*.

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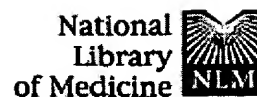
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Role of attached lipid in immunogenicity of *Borrelia burgdorferi* OspA.

Erdile LF, Brandt MA, Warakomski DJ, Westrack GJ, Sadziene A, Barbour AG, Mays JP.

Connaught Laboratories, Inc., Swiftwater, Pennsylvania 18370.

OspA is a protective antigen of the Lyme disease spirochete *Borrelia burgdorferi*. Expression of the full-length *B. burgdorferi* B31 OspA gene in *Escherichia coli* produces a protein that is processed posttranslationally by signal peptidase II and contains an attached lipid moiety. The recombinant OspA lipoprotein has been purified by detergent extraction and ion-exchange chromatography. Priming and boosting with OspA lipoprotein, either with no adjuvant or adsorbed to alum, elicited a strong, dose-dependent immunoglobulin G response. Serum from vaccinated mice inhibited spirochetal growth in vitro. Mice immunized twice with as little as 0.4 micrograms of OspA lipoprotein were protected against an intradermal challenge with 10(4) infectious spirochetes. The ability of the purified recombinant lipoprotein to induce a strong protective response in the absence of toxic adjuvants makes it an excellent candidate antigen for a human vaccine against Lyme disease. By contrast, no serum immunoglobulin G or growth inhibitory response to OspA nonlipoprotein was seen at any dose. The difference in immunogenicities of the lipoprotein and nonlipoprotein forms of OspA is not due to any difference in the antigenicities of the two proteins. These results suggest that posttranslational lipid attachment is a critical determinant of the immunogenicity of the OspA protein.

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The recombinant *Klebsiella pneumoniae* outer membrane protein OmpA has carrier properties for conjugated antigenic peptides.

Haeuw JF, Raully I, Zanna L, Libon C, Andreoni C, Nguyen TN, Baussant T, Bonnefoy JY, Beck A.

Centre d'Immunologie Pierre Fabre, Saint Julien en Genevois, France.
jean.francois.haeuw@pierre-fabre.com

Klebsiella pneumoniae OmpA, the 40-kDa major protein of the outer membrane, was cloned and expressed in *Escherichia coli*. The recombinant protein was produced intracellularly in *E. coli* as inclusion bodies. Fusion of a short peptide to the N-terminus of native P40 facilitated high-level expression of the recombinant protein. Purified recombinant P40 was analyzed to verify purity and structural integrity. The molecular mass of purified recombinant P40 determined by electrospray mass spectrometry was 37,061 Da, in agreement with the theoretical mass deduced from the DNA sequence. Specific proliferation of recombinant-P40-primed murine lymph node cells in response to recombinant P40 stimulation in vitro indicated the presence of a T-cell epitope on recombinant P40. The induction of high serum antibody titers to a synthetic peptide derived from the attachment protein G of the respiratory syncytial virus when chemically coupled to recombinant P40 indicated that the protein had potent carrier properties.

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Infect. Immun., 10 1994, 4632-4636, Vol 62, No. 10
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Biological activities of native and recombinant *Borrelia burgdorferi* outer surface protein A: dependence on lipid modification

JJ Weis, Y Ma and LF Erdile

Department of Pathology, University of Utah School of Medicine, Salt Lake City 84132.

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Borrelia burgdorferi lipoproteins are 50- to 500-fold more active as cytokine inducers and B-cell mitogens than *Escherichia coli* lipoproteins and synthetic peptides containing the tripalmitoyl-S- glyceryl-cysteine moiety. To investigate the source of this unique potency, we compared native OspA from *B. burgdorferi* with recombinant lipidated OspA produced in *E. coli*. As little as 10 ng of either protein per ml stimulated B-cell proliferation and production of cytokines and nitric oxide by macrophages. The two proteins induced comparable antibody responses in mice. Nonlipidated OspA made in *E. coli* had no stimulatory activity. Thus, lipid modification is essential both in vivo and in vitro for the immunological properties of OspA. The lipid moiety appears equally active whether produced in *B. burgdorferi* or in *E. coli*.

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